PATENT COOPERATION TREATY



# PCT

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>K 3155 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/EP 03/13413 | International filing date *(day/month/year)*<br>28.11.2003 | Priority date *(day/month/year)*<br>29.11.2002 |
| International Patent Classification (IPC) or both national classification and IPC<br>C07K14/47 | | |
| Applicant<br>DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG... et al | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 25.06.2004 | 24.03.2005 |
| Name and mailing address of the International preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized Officer<br>Fausti, S<br>Telephone No. +49 89 2399-7389 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/13413

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

| | |
|---|---|
| 1-4, 6-28 | as originally filed |
| 5 | filed with telefax on 07.03.2005 |

**Claims, Numbers**

| | |
|---|---|
| 1-16 | filed with telefax on 07.03.2005 |

**Drawings, Sheets**

| | |
|---|---|
| 1-5 | as originally filed |

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

   **see separate sheet**

6. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-16 |
| | No: | Claims | - |
| Inventive step (IS) | Yes: | Claims | 3-4 |
| | No: | Claims | 1-2,5-16 |
| Industrial applicability (IA) | Yes: | Claims | 1-16 |
| | No: | Claims | - |

2. Citations and explanations

   **see separate sheet**

**Re Item I**
**Basis of the report**

A. AMENDMENTS (Art. 34(2)(b) PCT).

A.1 With respect to the originally filed documents, claim 1 has been amended by introducing the feature of a cleavable spacer between the nuclear localization sequence and the transmembrane module. This amendment is supported by original claim 6, which discloses the preferred features of the conjugate of claim 1, among which the cleavable spacer. The introduction of only one of these preferred features in the definition of claim 1 does not contravene the requirements of the PCT because claim 6 discloses these preferred features both in combination and independently one from the others (see "and/or").

A.2 Claim 3 has been amended by introducing the structural feature of the homeobox protein derivative in terms of a minimal sequence identity, as disclosed on page 6 (see lines 2-3) of the originally filed patent specification. This amendment therefore meets the requirements of the PCT.

A.2[a] In addition, claim 3 has been amended by deleting the functional feature of the protein derivative (see "having... the same biological activity"). The omission of this functional feature corresponds to a generalization of the original disclosure, which is not "compensated" by the added structural feature, because not all the derivatives within the 60% of sequence identity necessarily have the same biological activity of the homeobox protein. In view of this generalization/omission, the subject-matter of amended claim 3 extends beyond the content of the application as originally filed and the requirements of Article 34(2)(b) PCT are not met.

A.2[b] For examination purpose, claim 3 has been considered as the functional feature would not be omitted, while the structural feature of the minimal sequence identity applies.

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicab citations and explanations supporting such statement**

1. DOCUMENTS.

The following documents are referred to in this communication:

D1: WO 95/34295;
D2: FEBS Letters (1989), vol. 244, no. 2, pages 439-446;
D3: Journal Of Molecular Biology (2002), vol. 318, no. 2, pages 237-243;
D4: Bioconjugate Chemistry (2000), vol. 11, no. 3, pages 301-305.

1.1 D1 discloses complexes for delivering a biologically active molecule into cell nucl comprising the biologically active molecule linked to an "importation competent si peptide" and a "nuclear localization peptide" (see claim 11). The signal peptide is selected and tested according to its ability to translocate across the cell membrar and is therefore to be considered a transport peptide (see page 11, first paragrap In preferred embodiments, the signal peptide is a fragment from the Kaposi fibrol growth factor (K-FGF) (see claim 12), and the nuclear localization peptide is a sequence from the acidic fibroblast growth factor (aFGF) or from the NF-κB p50 subunit (see claims 13-15).

1.2 D2 describes the characteristics of signal peptides from various species (see pa 439: left-hand column, second paragraph; right-hand column, last paragraph).

1.3 D3 discloses conjugates for the delivery of Peptide Nucleic Acids (PNAs), as model, to the nucleus of living cells (see abstract and table 1). These conjug comprise the amphiphilic transport peptide $pAntp_{(43-58)}$ from the Antennaper homeodomain of *Drosophila*, the NLS of sequence PKKKRKV from the S' antigen, and the PNA. In particular, the transport peptide is linked to the a disulphide bond, and the NLS binds to the PNA via a poly-Lys (see t conjugates 4 and 5).

1.4 D4 discloses conjugates of MR contrast agents for in vivo cell tracking or molecular sensing (see abstract). The conjugates comprise the paramagnetic nucleus carrying moiety linked to a transport peptide, i.e. HIV-Tat, and are effective for the cellular internalization of the diagnostic label: Fe-oxide or chelate-Gd (see: page 301, right-hand column, lines 5-8; page 302, left-hand column, lines 1-4; figure 3).

2. CLARITY (Art. 6 PCT).

2.1 Although claims 15 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of preferred features. In particular, claims 15 and 16 relate to the medical use of the conjugate of claim 1 in the treatment of tumours. The subject-matter of these claims differs in the specific therapeutic strategy, namely a chemotherapeutical or an GNTC intranuclear treatment. The aforementioned claims therefore lack conciseness and as such do not meet the requirements of Article 6 PCT.

3. NOVELTY (Art. 33(2) PCT).

3.1 The claimed subject-matter is novel because the prior art does not disclose any molecular conjugate comprising: (i) an amphiphilic transport peptide of human origin, (ii) a nuclear localization sequence and (iii) a signalling or drug-carrying module. For example, the complexes of D1 differ in that their importation competent signal peptide (representing the transport peptide) is not an amphiphilic peptide of human origin, e.g. it is an hydrophobic fragment of the Kaposi fibroblast growth factor in the closest embodiment of claim 12 (see point 1.1 above). D2 does not disclose any conjugate comprising (ii) and (iii) (see point 1.2 above). The conjugates of D3 and D4 differ in that the transport peptide is not of human origin, e.g. it is from *Drosophila* or HIV (see points 1.3 and 1.4 above). In addition, no nuclear localization sequence is present in the conjugates of D4.

4. INVENTIVE STEP (Art. 33(3) PCT).

4.1 Document D1, which is considered to represent the relevant state of the art, discloses complexes for delivering a biologically active molecule into the cell nuclei (see point 1.1 above), from which the subject-matter of claim 1 differs in that the "transmembrane module" is an "amphiphilic transport peptide of human origin", rather than an "importation competent signal peptide".

4.1[a] The problem to be solved may therefore be regarded as the provision of alternative conjugates, which comprise a transmembrane module, a nuclear localization sequence and the active module, for delivering said active module into cell nuclei.

4.1[b] The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step for the following reasons.

4.1[c] The subject-matter of claim 1 consists in the selection of an "amphiphilic transport peptide of human origin" from the range of possibilities, which are indicated in D1 by means of the reference to D2 (see page 11, lines 1-2). Among the possible choices for the (importation competent) signal peptide, D2 discloses signal peptides of human origin (see the references to "*Homo sapiens*" and "*Homo*"), which are characterised by a short, positively charged N-terminal region followed by a central hydrophobic region and a more polar C-terminal region, i.e. they are amphiphilic (see point 1.2 above and in particular: page 441, right-hand column, lines 3-5; page 442, right-hand column, lines 8-10; page 443, left-hand column, lines 1-4).
Contrary to the Applicant's/Representative's opinion, the skilled person would consider the disclosure of D2 in order to solve the problem posed in view of the explicit reference to this document contained in D1. In addition, D2 is considered to provide an enabling disclosure, from which the skilled person would have derived the claimed conjugate without the exercise of inventive skill. The disclosure of suitable signal peptide in D2 is not limited to the list of Table 1, but also concerns the peptides of human origin contained in the SIGPEP database (see: page 439, right-hand column, last paragraph). The specific amino acid sequences of the amphiphilic signal peptides of human origin are (were) therefore available to the skilled person in this database for the purpose of implementing the teaching of the prior art. Moreover, the subject-matter of claim 1 is not limited in terms of any specific amino acid sequence for the transmembrane module (i.e the amphiphilic transport peptide of human origin). Hence, the teaching from claim 1 is not superior to the one derived from the combination of D1 and D2, and no additional characterizing feature, to which an

inventive step could be addressed, is present in the definition of claim 1.

4.1[d] The selection of the transport peptide referred to in point 4.1[c] above can only be regarded as inventive, if it leads to unexpected effects or properties in relation to the other possibilities. No unexpected effects or properties are indicated in the application and therefore no inventive step is present in the subject-matter of claim 1.

4.1[e] The reduced antigenicity is not to be considered as an unexpected property since it can be generically expected that peptides derived from human proteins are less antigenic (or even no antigenic at all) to humans in comparison with protein fragments derived from other species.

4.1[f] Moreover, the skilled person would have likely selected, among the suggested signal peptides, the ones of human origin in view of the specific targeting effects that can thereby be achieved (see D1, page 11, lines 3-6).

4.2 Dependent claims 2, 5-11 do not appear to contain any additional features which, in combination with the features of any claim to which they refers, meet the requirements of the PCT with respect to inventive step.

4.2[a] With respect to claim 2, it is noted that conjugates of MR contrast agents and transport peptides for the intracellular delivery of paramagnetic Gd or Fe nuclei are disclosed in D4 (see point 1.4 above).

4.2[b] The preferred Nuclear Localization Sequence of claim 5, as well as the specific structures of the conjugate defined in claims 5-10, are suggested in D3 (see point 1.3 above).

4.2[c] The combination of a cytotoxic drug with such a conjugate is suggested in D1 (see page 6, lines 18-20).

4.3 The subject-matter of claims 3 and 4 is considered to involve an inventive step because the human homeobox protein HOX-B1 has not been suggested as a transport peptide in conjugates, which further comprise nuclear localization sequences and active modules.

4.3[a] Nevertheless, it is noted that the same conclusion does not apply to claim 3 if the functional feature of "the same biological activity" is not taken into account (see points A.2[a]-A.2[b] above). As not all the HOX-B1 derivatives within 60% of sequence identity have the relevant biological activity, they are not suitable as transport peptides (transmembrane module). Hence, the problem posed (see point 4.1[a] above) cannot be solved over the whole scope of broadened claim 3.

4.4 The subject-matter of any of claims 12-16 cannot be considered as involving any inventive step as long as the same applies to claim 1 because the conjugates of the prior art are also suggested for the same or similar medical uses (see in particular: D1, page 6, second paragraph; D3, page 242, left-hand column, lines 5-9; D4, abstract).

5. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

5.1 Claims 1-16 relates to pharmaceutical compounds and methods for the preparation of pharmaceutical compositions, which ban be made or applied in the pharmaceutical industry. Hence, the claimed subject-matter is to be considered industrially applicable according to Article 33(4) PCT.

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## Amended Claims

1. A conjugate comprising (a) an amphiphilic transport peptide of human origin as a transmembrane module (TPU), (b) a nuclear localization sequence (NLS), wherein said nuclear localization sequence is covalently coupled to the transmembrane module via a cleavable spacer, and (c) a signalling and/or drug carrying module (SM).

2. The conjugate of claim 1, wherein the signalling and/or drug carrying module comprises Gd, Ga, Mn, I, Fe and/or F as (diagnostic) image creating compound.

3. The conjugate of claim 1 or 2, wherein the transmembrane module (TPU) is the human homeobox protein HOX-B1 or a ~~fragment or~~ derivative thereof having an amino acid sequence identity to HOX-B1 of at least 60% ~~substantially the same biological activity~~.

4. The conjugate of claim 3, wherein the transmembrane module (TPU) comprises the amino acid sequence TQVKIWFQNRRMKQKK.

5. The conjugate according to any one of claims 1 to 4, wherein the nuclear localization sequence (NLS) comprises the amino acid sequence PKKKRKV or KPKRVKK.

6. The conjugate according to any one of claims 1 to 5, wherein ~~the transmembrane module (TPU) is coupled to the nuclear localization sequence (NLS) via a covalently cleavable spacer I and/or~~ the nuclear localization sequence (NLS) is coupled to the signalling and/or drug carrying module (SM) or a compound trapping the signalling and/or drug carrying module (SM) via a non-cleavable spacer II.

7. The conjugate according to claim 6, wherein spacer I

comprises a cleavable disulfide bridge.

8. The conjugate according to claim 7, wherein spacer II is polylysine.

9. The conjugate according to any one of claims 6 to 8, wherein spacer II carries an FITC label.

10. The conjugate according to any one of claims 1 to 9, wherein the conjugate has the following structure: transmembrane module (TPU) - spacer I - nuclear localization sequence (NLS) - spacer II - signalling and/or drug carrying module (SM) or compound trapping the signalling and/or drug carrying module + signalling and/or drug carrying module (SM).

11. The conjugate of any one of claims 1 to 10, wherein said conjugate further comprises a cytotoxic drug.

12. Use of the conjugate of any one of claims 1 to 10 for the preparation of a diagnostic composition for cell tracking.

13. Use of the conjugate of any one of claims 1 to 10 for the preparation of a contrast agent for MRI.

14. Use of the conjugate of any one of claims 1 to 10 for the preparation of a diagnostic composition for determining the activity of DNA repair enzymes.

15. Use of the conjugate of any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the chemotherapeutical treatment of a tumor.

16. Use of the conjugate of any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the intranuclear GNCT-treatment of a tumor.

transmembrane module (TPU), (b) a nuclear localization sequence (NLS) and (c) a signalling and/or drug carrying module (SM), preferably having Gd, Ga, I, Fe, Mn and/or F as image creating compound.

Methods for preparing the components of the conjugates of the present invention and for coupling are, e.g., disclosed in the German Patent Application No. 199 33 492.7. The transport mediator for the cell membrane (= transmembrane module (TPU)) is an amphiphilic transport peptide~~, preferably of human origin~~, which can penetrate the plasma membrane. The length of this peptide is not subject to any limitation as long as it has the above property. The cell nucleus addressed delivery system of the present invention is based on the cell immanent Ran/Karyopherine systeme. TPUs suitable for the conjugate of the present invention can be selected according to the methods described in Example 1, e.g., by searching for peptides of human origin containing sequence homologies to the sequence of the Antennapedia peptide fragment RQIKIWFQNRRMKWKK and analysing their capability to pass the cell membrane according to the methods described in Example 1. Examples of TPUs are derived preferably from the penetratin family (Derossi et al., Trends Cell Biol. 8: 84-87, 1998) or are transportan or parts thereof (Pooga et al., The Faseb Journal 12: 68, 1998). Particularly preferred examples of TPUs are derived from Penetratin 1, Antennapedia$^{1}$ $^{Hom}$[HoxB$_5$], TP$^{(1AOP/E.coli)}$, or PTD$^{TAT/HIV1}$. Further suitable TPUs are HBX5, HBX7 and HXD9. In a preferred embodiment, the transmembrane module (TPU) is the human homeobox protein HOX-B1 ~~or a fragment~~ or derivative thereof having the same biological activity, i.e. can still pass the cell membrane.

The term "derivative" in this context means that the amino acid sequences of these molecules differ from the sequences of the original molecule (HOX-B1) (due to substitution(s), addition(s) and/or deletion(s) of one or more amino acids) at one or several